Environment International 149 (2021) 106104

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Effects of prenatal exposure and co-exposure to metallic or metalloid elements on early infant neurodevelopmental outcomes in areas with small-scale gold mining activities in Northern Tanzania

Elias C. Nyanza [a,b], Francois P. Bernier [c,d,e], Jonathan W. Martin [f,i], Mange Manyama [g], Jennifer Hatfield [a], Deborah Dewey [a,c,d,e,h,*]

[a] Department of Community Health Sciences, Cumming School of Medicine, University of Calgary, 3280 Hospital Drive NW, Calgary, AB T2N 4Z6, Canada
[b] Department of Environmental, Occupational Health and GIS, School of Public Health, Catholic University of Health and Allied Sciences, P.O. Box 1464, Bugando, Mwanza, Tanzania
[c] Department of Paediatrics, University of Calgary, Alberta Children's Hospital, 28 Oki Drive NW, Calgary, AB T3B 6A8, Canada
[d] Department of Medical Genetics, Cumming School of Medicine, 3330 Hospital Drive NW University of Calgary, Canada
[e] Alberta Children's Hospital Research Institute, Cumming School of Medicine, University of Calgary, Room 294, Heritage Medical Research Building, 3330 Hospital Drive, NW Calgary, AB T2N 4N1, Canada
[f] Science for Life Laboratory, Department of Environmental Science, Stockholm University, 106 91 Stockholm, Sweden
[g] Division of Medical Education, Weill-Cornell Medicine-Qatar, Doha, Qatar
[h] Owerko Centre, #397 Child Development Centre, University of Calgary, 2500 University Dr. NW Calgary, AB T2N 1N4, Canada
[i] Department of Laboratory Medicine and Pathology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB T6G 2R3, Canada

ARTICLE INFO

Handling Editor: Shoji Nakayama

Keywords:
Lead, Mercury
Arsenic
Cadmium
Co-exposure
Artisanal and small-scale gold mining
Neurodevelopmental impairment

ABSTRACT

Background: Artisanal and small-scale gold mining (ASGM) is associated with release of neurotoxic metallic or metalloid chemical elements including lead (Pb), mercury (Hg), cadmium (Cd) and arsenic (As).
Objective: To examine associations between prenatal exposure and co-exposure to total lead (T-Pb), total mercury (T-Hg), total cadmium (T-Cd) and total arsenic (T-As) and infant neurodevelopment at 6 to 12 months of age in areas with ASGM activities in Tanzania.
Methods: Women in their second trimester of pregnancy who resided in ASGM areas were enrolled from 2015 to 2017 (n = 883). At 6 to 12 months of age, children were assessed with the Malawi Developmental Assessment Tool (MDAT) (n = 439). We measured T-Pb, T-Hg, and T-Cd in maternal dried blood spots and T-As in maternal urines. Poisson regression was used to examine associations between prenatal concentrations of these elements and neurodevelopmental outcomes.
Results: Prenatal T-Hg concentration was associated with global neurodevelopment status (aPR 1.03, CI:1.01–1.04; $p < 0.001$) and language impairment (aPR 1.05, CI:1.03–1.07; $p < 0.001$) on the MDAT. When prenatal T-Hg and T-As values were at or above the human biomonitoring reference values (≥95%) of the German Environmental Survey for Human Biomonitoring, that is 0.80 μg/L and 15 μg/L, respectively, the prevalence ratio of global neurodevelopmental impairment was two times higher (aPR 2.1, CI:1.0–4.3; $p = 0.034$). There was a 40% increase in the prevalence ratio of global neurodevelopmental impairment (aPR 1.4, CI:0.90–2.10, $p = 0.027$), when prenatal T-Hg was at or above the reference value of 0.80 μg/L and T-Pb was at or above the reference value of 35 μg/L. When prenatal T-Hg was at or above the reference value of 0.80 μg/L and T-As was at or above the reference value of 15 μg/L, the prevalence ratio of global neurodevelopmental impairment was two times higher (aPR 2.1, CI:1.0–4.3; $p < 0.034$).
Discussion: Infants born to women in areas with ASGM activities are at significant risk for neurodevelopmental impairment and this is associated with exposure to higher concentrations of Hg prenatally. Co-exposure to high concentrations of Hg and Pb, or Hg and As appeared to have negative potentiated effects on infants' neurodevelopment.

* Corresponding author at: #297 Owerko Centre, Child Development Centre, University of Calgary, 2500 University Dr. NW, Calgary, AB T2N 1N4, Canada.
E-mail address: dmdewey@ucalgary.ca (D. Dewey).

https://doi.org/10.1016/j.envint.2020.106104
Received 18 April 2020; Received in revised form 7 August 2020; Accepted 27 August 2020
Available online 16 September 2020

1. Introduction

Lead (Pb), mercury (Hg), cadmium (Cd) and arsenic (As) are neurotoxic metallic or metalloid chemical elements that are ubiquitous in the environment. They occur naturally but are also considered contaminants when their environmental concentrations are elevated due to anthropogenic activity. A growing body of evidence suggests that prenatal and early-life exposure to these elements is a public health concern. Many studies have reported that Pb can disrupt brain development, especially when exposure occurs during fetal life and in early childhood, having detrimental effects on neurodevelopment and behavior even at low concentrations (Lanphear et al., 2005; Grandjean and Landrigan, 2014). The neurotoxic effects of acute and chronic exposure to high concentrations of Hg are well documented (Harada 1995), but reports on the neurodevelopmental effects of prenatal exposure to methylmercury via the maternal diet have been mixed (Barbone et al., 2019; Davidson et al., 2011; Debes et al., 2016; Oken et al., 2005; Xu et al., 2016). Moreover, little is known about the neurodevelopmental effects of prenatal exposure to elemental mercury from activities such as artisanal and small-scale gold mining (Bose-O'Reilly et al. 2010). Limited research has suggested that early life exposure to Cd is associated with poorer social and cognitive functioning in young children (Gustin et al., 2018; Kippler et al., 2012). Finally, prenatal exposure to As from drinking water has been associated with cognitive impairments among children as young as five years of age (Tolins and Ruchirawat, 2014; Tyler and Allan, 2014).

Exposure to metallic and metalloid elements rarely occurs in isolation and co-exposure is likely the norm. Studies investigating the interactive effects of co-exposure to these elements on neurodevelopmental outcomes are limited (Freire et al., 2018; Kim et al., 2013; Sanders et al., 2015; Valeri et al., 2017). Kim et al (2013) found an antagonistic dose-dependent interaction between co-exposure to Pb and Cd in early pregnancy (<20 weeks gestation) and cognitive development at 6 months of age. Freire et al. (2018) reported a synergistic effect between prenatal Pb and As concentrations in placenta tissue with respect to general cognitive performance at 4–5 years of age, but an antagonistic interaction between Hg and manganese on the same population. Valeri et al. (2017) reported that prenatal exposure to As, manganese and Pb negatively affected cognitive scores at 20 to 40 months of age when blood metal concentrations were above the 60th percentile.

Much of the research investigating the effects of prenatal exposure to neurotoxic elements has been conducted in high income countries; however, the prevalence and magnitude of exposure can be higher in economically deprived areas of the world (Grandjean et al., 2015). Artisanal and small-scale gold mining (ASGM) is a global activity conducted mainly in low- and middle-income countries and has been associated with the release of neurotoxic metallic and metalloid elements into the environment (Nyanza et al. 2014). Individuals in ASGM communities, including women of reproductive age and children, are exposed neurotoxic metallic elements through various pathways including the water they drink, food they eat, air they breathe, and soil in which their food is grown (Nyanza et al., 2014; UNEP, 2013). These elements can accumulate in maternal blood and cross the placental barrier and could have significant long-lasting effects on neurodevelopment (Grandjean and Landrigan, 2014; Sanders et al., 2015). This study investigated the associations between maternal co-exposure to total Pb, Hg, Cd, and As during pregnancy and infant neurodevelopment in areas with ASGM activities in Tanzania. We hypothesized that higher prenatal co-exposure to these chemical elements would be associated with adverse neurodevelopment.

2. Methods

2.1. Study design and participants

This study is part of the ongoing Mining and Health prospective longitudinal study in Northern Tanzania. Between 2015 and 2017, women in their second trimester of pregnancy who had resided in areas with ASGM activities for more than six months were recruited during antenatal care clinic visits and followed until pregnancy outcome was registered. A total of 905 women consented to participate in the study, but 22 failed to complete the scheduled interview or bioindicator sampling. In addition, women with an adverse birth outcome (n = 184), no record of birth outcome (n = 95), a multiple birth (n = 2) or whose infant was identified with a visible congenital anomaly (n = 12) were excluded from the study (Nyanza et al. 2020). An additional 151 women were lost to follow-up. This resulted in a sample of 439 mother–child pairs (Figure S1). Maternal blood concentrations of Pb, Hg, and Cd and urine concentrations of As were measured during the second trimester of pregnancy and infants participated in a neurodevelopmental assessment between 6 and 12 months of age (infant mean age 7.92 ± 1.77 months). The Catholic University of Health and Allied Sciences and Bugando Medical Centre's Joint Ethics and Research Review Committee (BREC/001/38/2014), University of Calgary Conjoint Health Research Ethics Board (REB14-2051), and Tanzania National Institute for Medical Research (MR/001/38/2014) approved the study. Permission was obtained from relevant health authorities at the regional and district levels in Geita. Pregnant women over 18 years of age provided written informed consent; pregnant women younger than 18 years of age provided assent, and their parents or guardians provided informed consent. If women had low literacy levels, a witness read the consent form and a thumbprint indicating consent was obtained (Nyanza et al. 2019a).

2.2. Maternal Lead, Mercury, cadmium and arsenic concentrations

Dried blood spots (DBS) were used to measure total lead (T-Pb), total mercury (T-Hg), and total cadmium (T-Cd) concentrations, while maternal spot urine samples (~20 mL) were used to measure total arsenic (T-As) concentrations. DBS and spot urine samples were collected on the same day after a face-to-face interview was completed with the participant. All samples were collected between 16 and 27 weeks gestational age. Spot urine samples were collected in acid washed polyethylene containers and preserved with 1% hydrochloric acid and stored below −8°C prior to shipment to the laboratory to prevent bacteria growth and absorption of the analytes. For DBS collection, a drop of capillary blood was collected on filter paper (Whatman #903) following a simple finger prick as previously described and validated (Nyanza et al. 2019b). To ensure the validity and reliability of the DBS results, quality control measures were instituted during sample collection and processing (see Nyanza et al., 2019a; 2019b). Briefly, field and laboratory blanks (i.e., blank Whatman #903 filter papers) were integrated into sampling and analytical procedures to account for possible contamination. To avoid exposure misclassification, method detection limits (MDLs) (0.08, 0.012, and 0.004 μg/L for Pb, Hg, and Cd, respectively) were determined based on field blanks (i.e., mean + 3 times standard deviation). Also, for every batch of 10 samples analyzed, Laboratory Seronorm reference material (SRM), L-2®, (from Sero AS, Billingstad, Norway) were run to assess the accuracy of the analytical procedure. In addition, after every 20 DBS samples, a replicate second sample from the same participant was analyzed under identical laboratory conditions. The DBS replicate mean analytical precision was<10% relative standard deviation. DBS samples were also compared to venous blood samples from the same participant, and among 44 participants a strong correlation ($r^2 > 0.9$), and a slope close to 1.0 between DBS concentration and quantitative venous blood indicated high accuracy of the method (Nyanza et al. 2019b).

The DBS and urine samples underwent microwave-assisted closed

vessel acid digestion at 170 °C for 30 min. All punched DBS and blank filter paper samples were adjusted to a volume of 5 mL after digestion. To determine chemical element concentrations from DBS, the mass of dry blood in each spot was first measured. This was done by subtracting a blank filter paper punch from the same lot from the dried blood spot punch. The range of dried blood mass corresponded to 45 to 55 μL of wet whole blood (Nyanza et al. 2019b). For field blanks, a volume of 50 μL of blood was assumed (Li et al. 2014 Nyanza et al. 2019b). The concentration of chemical elements in the sample and filter blank digests were determined by inductively coupled plasma mass spectrometry (ICP-MS; PerkinElmer, Shelton, CT, USA) (μg/L) (Chaudhuri et al. 2009). Multiple isotopes were monitored for each of the chemical elements and all results were within 10% relative standard deviation, indicating precision of the chemical analysis (Nyanza et al. 2019b). This analysis was completed at an ISO 17025 accredited laboratory (ALS Scandinavia, Sweden) (Nyanza et al. 2019b).

2.3. Infant neurodevelopment assessment

The Bayley Scales of Infant Development (BSID) have been used to assess the developmental outcomes of children exposed to methylmercury and arsenic (e.g., Davidson et al., 1995; Tofail et al., 2009). However, tools developed and validated in Western countries, such as the BSID, can provide misleading findings in different cultural settings where some items are unfamiliar and standardized norms for local pediatric populations are not available. Therefore, in the current study, we used the Malawi Developmental Assessment Tool (MDAT), a culturally appropriate tool, to assess infant neurodevelopment between 6 and 12 months of age (Gladstone et al. 2010). The MDAT has been validated for use in children up to six years of age in rural sub-Saharan Africa (Gladstone et al. 2010). and has been shown to have excellent internal reliability (Kappa $>$ 0.75 for 99%) and good sensitivity (97%) and specificity (82%) (Gladstone et al. 2010). It assesses four domains of function: gross motor, fine motor, language and social. In the present study, alpha coefficients for the items in the gross motor, fine motor, language, and social were 0.91, 0.89, 0.91, and 0.94 respectively. The MDAT was translated into Kiswahili language and pre-tested prior to study initiation.

Public health nurses at local health facilities were trained to administer the MDAT according to procedures outlined in the MDAT administration manual (Gladstone et al. 2010). Briefly, the nurses were provided information on child development from infancy to 6 years of age and on ethical issues related to the conduct of research with children (Gladstone et al., 2010; Dewey et al., 2018). This was followed by a session that provided training and practice in calculating children's chronological ages and administrating the assessment measure. To increase the reliability of the assessment and to reduce potential biases, two public health nurses participated in and scored the assessment for each child; after each assessment the nurses compared their scores on each item and reconciled any scores where a difference was noted through discussion (Gladstone et al. 2010). The nurses who conducted the assessments were blinded to the prenatal exposure concentrations of the children.

For each domain, a total score was computed by summing the number of items that a child passed at the 90% percentile level for their age. The scores in each domain could range from 0 to 34. Children's scores were classified as follows: 1) normal outcome if they performed $\geq$ 90th percentile level on all of the items in that domain or $<$ 90th percentile on one or two items in the domain; or 2) impaired if they performed $<$ 90th percentile on more than two items in a domain. Infants were also classified in terms of global neurodevelopment status; infants who displayed a normal outcome on all domains were classified as normally developing and infants who were impaired on at least one domain were classified as displaying neurodevelopmental impairment.

2.4. Covariates

Covariates were selected based on research evidence that indicated a potential influence on prenatal maternal concentrations of chemical elements (Nyanza et al. 2019a) or infant neurodevelopmental outcomes (Kim et al., 2013; Freire et al., 2018). Structured self-report questionnaires administered during pregnancy and follow-up (at birth and at 6 to 12 months of age) were used to obtain information on maternal age at birth, maternal education, maternal and paternal occupation, number of under-five siblings at home, and family socioeconomic status. In rural low-income settings, an individual's income is difficult to determine (Vyas and Kumaranayake 2006). Traditional indicators, similar to those used in Demographic and Health Surveys (TDHS-MIS 2017), which measure asset ownership (e.g., ownership of house or bicycle, home sanitation facilities), were used to estimate participants' socioeconomic wealth quintiles (SEWQ) (Vyas and Kumaranayake 2006). Data on infant sex, age, birthweight, height and weight were obtained at the neurodevelopmental assessment. Birthweight was categorized as low (i.e., $<$2500 g) or normal (i.e., $\geq$2500 g). Since nutritional status has an influence on child development (UNICEF-WHO, 2012), we measured height and weight at the time of the assessment as a proxy for nutritional status and compared it to the World Health Organization (WHO) reference population norms for children (WHO 2010). Cut-off Z-scores and their standard deviations from the WHO norms were used to classify children's nutritional status according to three indices (i.e., underweight: Z-score $<$ -2SD from the median weight for age; stunting: $<$-2SD from the median weight for height; wasting: $<$-2SD from the median height for age) (Anthro for personal computers, version 3.2.2. WHO Geneva, Switzerland, 2010).

2.5. Statistical analysis

Statistical analyses were performed using Stata version 14.1 (Stata Corp LP®, College Station, Texas, USA). Findings were summarized as means or medians with their corresponding measures of dispersion for continuous variables; frequencies with their respective percentages were used for categorical variables. Poisson regression models were used to estimate associations between prenatal concentrations of Pb, Hg, Cd, and As, selected covariates and neurodevelopmental outcomes. All of the covariates were retained in the univariate analysis to avoid the loss of potential confounders and were included in the multivariate regression model to build a core model. In the multivariable models, neurodevelopmental impairment was adjusted for all the covariates studied. Covariates showing a significant association with neurodevelopmental outcome at $p < 0.20$ in the final multivariate regression models are presented on Table 5. In all cases, inferences were made using 95% confidence intervals. A p-value of $p < 0.05$ was considered statistically significant.

Since neurodevelopmental impairment was common (prevalence $\geq$ 10%), modified Poisson regression was used to estimate the prevalence ratios (PRs) and their 95% confidence intervals (CI). Robust standard errors were used in the Poisson regression models to ensure that the error for the estimated relative risk would not be overestimated (Chen et al., 2018; Tamhane et al., 2016; Zou, 2004). This is the preferred method for estimating predictors when utilizing logistic models and has been found to be appropriate when the prevalence of the outcome is common (Chen et al., 2018; Tamhane et al., 2016;).

Prenatal metallic or metalloid chemical elements were investigated for interaction effects on neurodevelopmental outcome. Spearman correlation coefficients were used to assess the relationships between T-Pb, T -Hg, T-Cd and T-As concentrations. To investigate the effects of co-exposures to T-Pb, T-Hg, T-Cd, and T-As, maternal prenatal concentrations were treated as dichotomous (i.e., those that were below the reference values versus those that were at or above the reference values).

Reference values are derived from population-based studies and can be used to classify measured levels of chemical elements in individuals

or population as elevated or not elevated. They are established based on statistically derived values indicating the upper margin of background exposure (95th centile) to a given chemical element in a given population at a given time (Schulz et al. 2009). Maternal human biomonitoring reference values for Pb, Hg, Cd and As have not been established and there are no established reference values for Tanzania or other Sub-Saharan Africa countries (Nyanza et al. 2019a). The developing fetus is far more sensitive to toxic chemical exposure than adults and some of developmental processes, such as cognition may be particularly vulnerable (Landrigan & Goldman, 2011). Therefore, we chose to compare the prenatal levels of T-Pb, T-Hg, T-Cd, and T-As among pregnant women in this study to the lowest human biomonitoring reference values for children that are presently available. These were established by the German Environmental Survey for Human Biomonitoring (GerEsIV) (Schulz et al. 2009); 35.0, 0.80, 0.30, and 15.0, μg/L for T-Pb, T-Hg, T-Cd, and T-As, respectively.

3. Result

3.1. Characteristics of the study participants

Comparison of participants who participated in neurodevelopmental assessments and those who were lost to follow-up revealed no significant group differences in SEWQ ($\chi2 = 5.37$, $p = 0.068$), maternal age ($\chi2 = 29.92$, $p = 0.572$), maternal education ($\chi2 = 0.989$, $p = 0.320$), maternal occupation ($\chi2 = 3.36$, $p = 0.186$), paternal occupation ($\chi2 = 0.$ 1.38, $p = 0.501$), presence of under-five siblings in the home ($\chi2 = 0.3.46$, $p = 0.063$), child sex ($\chi2 = 0.597$, $p = 0.785$), birth outcome ($\chi2 = 0.485$, $p = 0.466$), place of delivery ($\chi2 = 2.19$, $p = 0.334$), gestational age at birth ($\chi2 = 20.47$, $p = 0.275$), or birth weight ($\chi2 = 42.74$, $p = 0.051$). The descriptive characteristics of the families and children who participated (n = 439) in the present study are presented in Table 1.

Table 1
Family and infant characteristics (N = 439).

Family characteristics	N	(%)
Mothers median age at enrollment (years) (Median: 25, IQR: 20–30)		
Age group of mothers		
14–24	210	(47.8)
25–34	175	(69.7)
35+	54	(12.3)
Socioeconomic wealth quintile		
Good or High (>9)	65	(14.8)
Moderate (6–9)	224	(51.0)
Low (<6)	150	(34.2)
Mother's education		
No formal education	94	(21.4)
At least primary education	345	(78.6)
Mother's occupation		
Farm/Animal keeping	381	(86.8)
Mining	27	(6.2)
Business &Public servant	31	(7.0)
Paternal occupation		
Farm/Animal keeping	263	(59.9)
Mining	130	(29.6)
Business & Public Servant	46	(10.5)
Presence under-fives in home		
Yes	346	(78.8)
No	93	(21.2)
Infant Characteristics		
Mean age at assessment (months)(mean: 7.9 ± 1.8)		
Sex		
Male	213	(48.6)
Female	225	(51.4)
Place of birth		
Health facility	297	(68.1)
Home	139	(31.9)
Median gestational age at birth (weeks)(median: 37, IQR: 28–37)		
Birth weight		
<2500 gm	72	(16.4)
≥ 2500 gm	367	(83.6)
Median weight for age score (median: 0.50, IQR: −0.3–1.2)		
Underweight		
Yes	10	(2.3)
No	423	(97.7)
Median height for age score (median: −3.4, IQR: −4.7–1.7)		
Stunted		
Yes	323	(73.6)
No	116	(26.4)
Median weight for height score(median: 3.2, IQR: 1.8–4.8)		
Wasted		
Yes	15	(3.4)
No	424	(96.6)

Note: *Underweight – (i.e., Z-score < -2SD from the median weight for age of the WHO reference population); Stuned – (i.e., Z-score < -2SD from the median height for age of the WHO reference population); Wasted – (i.e., Z-score < -2SD from the median weight for height of the WHO reference population) (Anthro for personal computers, version 3.2.2. WHO Geneva, Switzerland, 2010).*

3.2. Maternal prenatal concentrations of metals and metalloids

Median concentrations of the four analytes were 27.2 (IQR = 17.2–42.5), 1.2 (IQR = 0.8–1.7), 0.20 (IQR = 0.2–0.3), and 8.3 (IQR = 4.5–14.9) μg/L, for T-Pb, T-Hg, T-Cd, and T-As, respectively (Table 2). For T-Hg, the majority of prenatal samples (n = 336, 76.5%) were above the GerEsIV reference value of 0.80 μg/L (Schulz et al. 2009). For T-Pb (n = 275, 62.6%), T-Cd (n = 370, 84.3%), and T-As (n = 331, 75.4%) most of the samples were below the GerEsIV reference values. Spearman correlations revealed significant positive correlations between T-Cd and T-Pb ($\rho = 0.26$, $p < 0.001$). No significant correlations were found between any other chemical elements (Figure S2).

3.3. Neurodevelopmental outcome of infants

Based on global neurodevelopment status classification, almost one-third of the infants (31.0%) met criteria for neurodevelopmental impairment. On specific domains of the MDAT, 13.9% (n = 61) were classified as impaired in gross motor skills, 15.3% (n = 67) in fine motor skills, 15.5% (n = 68) in language skills, and 6.8% (n = 30) in social skills (Table 3).

3.4. Multivariable regression analyses

Unadjusted univariate analyses revealed that prenatal T-Hg concentration was associated with global neurodevelopmental status and language skills (Table S1). In adjusted models, prenatal T-Hg concentration was significantly associated with global neurodevelopment status (Table 4); for a 1 μg/L increase in blood T-Hg, the prevalence ratio for global neurodevelopment impairment increased by 3% (aPR 1.03, CI:1.01–1.04; $p < 0.001$). T-Hg was also associated with a significantly higher prevalence ratio for language impairment; for every 1 μg/L increase of T-Hg in maternal prenatal blood, the prevalence ratio of language impairment increased by 5% (aPR 1.05, CI:1.03–1.07; $p < 0.001$).

Stratification analyses by sex revealed significant associations between increased prenatal T-Hg exposure, and language impairment (boys: aPR 1.05 (1.04–1.07), $p < 0.001$; girls: aPR 1.21 (1.16–1.27), $p < 0.001$) and global neurodevelopmental impairment (boys: aPR 1.03 (1.02–1.048), $p < 0.001$; girls: aPR 1.10 (1.06–1.14), $p < 0.001$) (Table S2). Female infants displayed an increased risk of social impairment with increased T-As levels (aPR 1.01 (1.00–1.02), $p < 0.05$), whereas male infants displayed increased risk of global neurodevelopmental impairment with increased prenatal T-Cd levels (aPR 2.55 (1.33–4.87), $p < 0.05$).

3.5. Individual and Co-Exposure effects and global neurodevelopmental impairment

Using the reference values established by the GerEsIV, the

Table 2
Maternal prenatal blood concentrations of T-Pb, T-Hg, T-Cd and urinary concentrations of T-As among participants (N = 439).

Prenatal Exposure Concentrations*(μg/L)	N	(%)	P10	P25	Median (95% CI)	P75	P95	Max
Median T-Pb			11.6	17.2	27.2 (25–30.3)	42.5	85.8	145
< 35	275	(62.6)						
≥ 35	164	(37.4)						
Median T-Hg			0.60	0.80	1.2 (1.1–1.3)	1.7	3.8	72.0
< 0.8	103	(23.4)						
≥ 0.8	336	(76.5)						
Median T-Cd			0.14	0.21	0.20 (0.20–0.21)	0.30	0.50	1.5
< 0.3	370	(84.3)						
≥ 0.3	69	(15.7)						
Median T-As			2.8	4.5	8.3 (7.4–9.3)	14.9	38.2	21.3
< 15	331	(75.4)						
≥ 15	108	(24.6)						

*We dichotomized T-Pb, T-Hg, T-Cd, and T-As based on human biomonitoring reference values established by the GerESIV of 35.0 μg/L, 0.80 μg/L, 0.30 μg/L, for blood lead, mercury, and cadmium, and 15 μg/L for urinary arsenic (Schulz et al. 2009). CI, confidence interval; ug/l, microgram/liter; P, percentile; T-Pb, total lead; T-Hg, total mercury; T-Cd, total cadmium; T-As, total arsenic.

Table 3
Children meeting the criteria for normal or impaired neurodevelopment on the MDAT (N = 439).

Developmental Outcome	Normal		Impaired	
	N	(%)	N	(%)
Global Neurodevelopmental status	303	(69.0)	136	(31.0)
Gross motor	378	(86.1)	61	(13.9)
Fine motor	372	(84.8)	67	(15.3)
Language skills	371	(84.5)	68	(15.5)
Social domain	409	(93.1)	30	(6.8)

prevalence ratio of global neurodevelopmental impairment was 80% greater for infants prenatally exposed to T-Hg at or above the reference level of 0.80 μg/L (aPR 1.8, CI:1.3–2.4; p < 0.001). When prenatal concentrations of T-Pb were at or above the reference value, the prevalence of global neurodevelopmental impairment increased by 20% (aPR 1.2, CI:1.01–1.4; p = 0.037). No significant effects were noted for T-Cd and T-As. Examination of co-exposure interactions revealed that the prevalence ratio of global neurodevelopmental impairment was 2 times higher (aPR 2.1, CI:1.0–4.3; p < 0.034) when prenatal T-Hg was at or above the reference value and T-As was at or above the reference value of 15 μg/L. The interaction between T-Hg and T-Pb revealed a 40% increase in the prevalence ratio of global neurodevelopmental impairment (aPR 1.4, CI:0.90–2.10, p = 0.027), when prenatal T-Hg was at or above the reference value and T-Pb was at or above the reference value of 35 μg/L. No other significant interactions were predictive of global neurodevelopmental outcomes (Table 5).

4. Discussion

This study revealed that many infants born to women living in areas with ASGM activities in Northern Tanzania are exposed to higher than recommended concentrations of Pb, Hg, Cd, and As during gestation. Higher concentrations of exposure to Hg were associated with a higher risk of global neurodevelopmental impairment and language impairment. Prenatal exposure to Pb and Hg at or above the reference values established by the GerEsIV was associated with a significantly increased prevalence of global neurodevelopmental impairment. Examination of the effects of co-exposures revealed that prenatal T-Hg exposure concentrations at or above the reference level of 0.80 μg/L were associated with global neurodevelopmental impairment among infants during the first year of life in the presence of either T-As or T-Pb at or above their reference values of 15 and 35 μg/L respectively. Thus, while As and Pb alone were not associated with poorer neurodevelopment, when combined with Hg they potentiated the adverse effects of Hg on neurodevelopmental outcomes.

The results of the present study support our hypothesis that prenatal exposure to metallic or metalloid elements in areas with ASGM activities is associated with neurodevelopmental impairment among infants. Our findings of associations between higher concentrations of prenatal exposure to Hg and global neurodevelopmental impairment and

Table 4
Adjusted associations between neurodevelopmental status (i.e., impaired versus normal) and exposure to lead, mercury, arsenic or cadmium and covariates (with p-value < 0.20) using multivariable Poisson regression analysis (adjusted prevalence ratio - aPR).

Variables	Gross motor aPR (95% CI)	Fine motor aPR (95% CI)	Language aPR (95% CI)	Social status aPR (95% CI)	Global neurodevelopmental status aPR (95% CI)
T-Pb (μg/L)	1.0 (0.9, 1.0)	1.0 (0.9, 1.0)	1.0 (1.0, 1.0)	1.01 (1.0, 1.02)	1.0 (0.9, 1)
T-Hg (μg/L)	0.9 (0.9, 1.1)	1.02 (0.9, 1.1)	1.05 (1.03, 1.07) **	0.9(0.8, 1.1)	1.03 (1.01, 1.04) **
T- Cd (μg/L)	0.9 (00.13–2.4)	2.0 (0.3, 13.0)	1.4 (0.5, 3.7)	0.3(0.1, 1.8)	0.8(0.4, 1.9
T-As (μg/L)	0.9 (0.9–1.0)	1.0 (0.9, 1.01)	1.1 (0.9, 1.0)	1.0 (0.9, 1.01)	1.0 (0.9, 1.0)
Socioeconomic wealth quintile					
High (>9)	1.2 (0.6, 2.2)	0.7 (0.5, 1.2)	0.7 (0.3, 1.6)	1.2 (0.7, 2)	1.1 (0.9, 1.4)
Moderate (6–9)	Ref	Ref	Ref	Ref	Ref
Low (<6)	0.7 (0.4, 1.2)	0.7 (0.5, 1.9)	1.1 (0.6, 1.9)	0.6 (0.3, 1.9)	0.7 (0.6, 1.9)
Infant age at assessment (months)	1.3 (1.1, 3.4)	1.5 (1.4, 1.6) **	0.11 (0.1, 0.2) **	1.1 (1, 2.7) *	0.5 (0.5, 0.6) **
Low birth weight					
Yes (<2500 gm)	0.5 (0.2, 1.9)	1.7 (1.2, 2.3) **	1.4 (0.5, 3.4)	1.3 (0.5, 3.3)	0.6 (0.3–1.4)
No (≥2500 gm)	Ref	Ref	Ref	Ref	Ref
Sex of infant					
Male	Ref	Ref	Ref	Ref	Ref
Female	1.7 (1.1–2.5) **	1.3 (0.8, 2.1)	0.9 (0.6, 1.5)	1. 3(0.8, 2.2)	1.4 (0.9, 2.1)

Note: T-Pb, T-Hg, T-Cd and T-A were added into the final models as locked terms despite their levels of statistical significance. The models were adjusted for all covariates. aPR = adjusted prevalence ratio; CI, confidence interval; gm, grams; T-Pb, total lead; T-Hg, total mercury; T-Cd, total cadmium; T-As, total arsenic; μg/L, microgram/liter. *p < 0.05; **p < 0.001

Table 5
Individual and co-exposure effects of prenatal exposure to Pb, Hg, Cd and As above Germany Environmental Survey IV reference levels.

Interaction Models	Global Neurodevelopmental Status		
	aPR	(95% CI)	p-value
Effect of T-Pb ≥ 35 (μg/L)	1.2	(1.01–1.4)	0.037
T-Hg ≥ 0.80	1.1	(0.9–1.4)	0.277
T-Cd ≥ 0.30	1.01	(0.7–1.5)	0.472
T-As ≥ 15	1.3	(0.9–1.8)	0.246
Effect of T-Hg ≥ 0.8(μg/L)	1.8	(1.3–2.4)	<0.001
T-As ≥ 15	2.1	(1.0–4.3)	0.034
T-Cd ≥ 0.30	2.9	(0.5–18)	0.074
T-Pb ≥ 35	1.4	(0.9–2.1)	0.027
Effect of Cd ≥ 0.3 (μg/L)	0.9	(0.7–1.1)	0.349
T-As ≥ 15	0.8	(0.5–1.2)	0.225
T-Hg ≥ 0.80	0.9	(0.7–1.1)	0.374
T-Pb ≥ 35	0.8	(0.6–1.1)	0.094
Effect of T-As ≥ 15 (μg/L)	1.02	(0.8–1.2)	0.707
T-Hg ≥ 0.80	1.04	(0.8–1.3)	0.969
T-Cd ≥ 0.30	0.9	(0.6–1.5)	0.351
T-Pb ≥ 35	1.1	(0.8–1.4)	0.900

Note: The interaction models were simultaneously adjusted for all the chemical elements and interaction terms in the regression model. aPR = adjusted prevalence ratio; CI, confidence interval; gm, grams; T-Pb, total lead; T-Hg, total mercury; T-Cd, total cadmium; T-As, total arsenic; μg/L, microgram/liter.

language impairment are consistent with those of a recent study in Spain that reported that higher concentrations of prenatal exposure to Hg based on placental concentrations were associated with poorer verbal functions in children at 4 to 5 years of age (Freire et al. 2018). Similarly, a study in the USA reported associations between higher concentrations of prenatal Hg exposure and poorer cognitive and psychomotor skills in infants at six months of age (Oken et al. 2005). The total prenatal mercury concentrations in the current study (median 1.2, Range 0.80 – 1.7 μg/L) are relatively elevated compared to those reported by Freire et al.(2018) in Spain (Median 0.025, Range 0.016 – 12.95 μg/L), and by Oken et al (2005) in the USA (mean 0.55, Range 0.02 – 2.38 μg/L). However, the studies of Freire et al. (2018) and Oken et al. (2005) were carried out in non-ASGM communities where exposure to Hg was mainly from fish consumption.

Despite this converging evidence, some studies have reported contradictory findings. Studies conducted in Italy and the United Kingdom that examined the effects of prenatal Hg exposure from ingested seafood have reported no association between Hg concentrations and impaired child neurodevelopment between 6 and 42 months of age (Deroma et al., 2013; Golding et al., 2016). It is important to note that fish and seafood contain nutrients such as vitamin D, choline and docosahexaenoic acid (DHA) (Wu et al. 2013) that are important for brain development (Zeisel, 2006; Lauritzen et al., 2016). Fish and seafood consumption have also been associated with improved neurodevelopment (Daniels et al., 2004; Oken et al., 2008; Nyaradi et al., 2013). As a result, nutrients associated with fish consumption could confound the effects of low dose prenatal Hg exposure on children's neurodevelopment (Oken et al. 2008). However, in the present study, we did not document fish consumption among participants.

The finding that prenatal Pb concentrations at or above the reference values of 35 μg/L were associated with impaired global neurodevelopment supports previous reports that have linked prenatal Pb exposure to communication deficits, hyperactive behaviour and attention deficits at 6 months (Jedrychowski et al. 2007) and 2 to 10 years (Schnaas et al., 2006; Wasserman et al., 2007). These findings support the need for actions that minimize Pb exposure in ASGM areas of Tanzania.

In previous research, we recommended the avoidance of geophagy practices (i.e., soil eating) during pregnancy as the soil and the soil sticks sold in local markets have been found to contain significant amounts of Pb (Msoffe et al. 2018). Reducing or eliminating such practices during pregnancy could result in lower concentrations of prenatal exposure to Pb and possibly other chemical elements among infants of women living in ASGM areas and potentially result in better neurodevelopmental outcomes.

In contrast to previous research (Hamadani et al., 2010, 2011; Parajuli et al., 2013; Roy et al., 2011; Von Ehrenstein et al., 2007; Wasserman et al., 2007), we did not find any associations between higher concentrations of prenatal exposure to As and neurodevelopmental impairment (except for female infants on the Social domain of the MDAT when stratifying by sex). This discrepancy could be because the children in our cohort were very young, 6–12 months of age, unlike most previous research, which assessed developmental outcomes between 5 and 15 years. Studies that have examined the effects of prenatal exposure to As on neurodevelopment have typically reported that associated problems are not evident until later childhood (Hamadani et al., 2010, 2011; Parajuli et al., 2013; Parvez et al., 2011; Roy et al., 2011). Also, previous studies in As endemic areas in Bangladesh, Taiwan, and West Bengal, India, have investigated neurodevelopmental outcome in communities exposed to high concentrations of As, above 50 μg/L from drinking water (Wasserman et al., 2007; Von Ehrenstein et al., 2007). In a previous study in these ASGM communities in Tanzania, As concentrations in drinking water were found to have a mean concentration of 10.5 μg/L (Nyanza et al. 2014), which is much lower. Therefore, it is possible that the neurodevelopmental effects associated with prenatal As exposure are associated with higher concentrations than those found in the present study. Future longitudinal studies are needed to determine if early exposure to As in ASGM areas is associated with children's neurodevelopmental status later in childhood.

Our finding of no association between Cd and neurodevelopmental outcomes (except for male infants on global developmental impairment when stratifying by sex) is consistent with much of the previous research (Gustin et al., 2018; Jeong et al., 2015; Rodriguez-Barranco et al., 2013; Sanders et al., 2015). However, a study by Kim et al (2013) reported an association between higher levels of prenatal cadmium exposure and children's neurodevelopment. Therefore, further research is needed to clarify the effect of prenatal exposure to Cd on children's long-term neurodevelopmental outcomes.

Even though a chemical element may not be associated with significant neurodevelopmental effects on its own, the interactive effect with another element could have an unanticipated mixture effect (i.e., additive, synergistic, antagonistic or potentiated) on children's neurodevelopment. We found no evidence that prenatal maternal exposure to either Pb or As on their own was associated with children's neurodevelopment, but when women with high levels of exposure to Hg (i.e., at or above reference values), were co-exposed to high levels of either Pb or As (i.e., at or above reference values), the adverse effects on neurodevelopment were potentiated (i.e., worsened). These findings are consistent with Boucher et al. (2012) who reported a significant synergistic Pb-Hg interaction, where high Pb with increasing Hg concentrations, was associated with cognitive impairment among 9 to 13 years old Inuit children living in Quebec, Canada. In contrast, an antagonistic Pb-Hg interaction was reported among children 7 to 14 years of age in the Faroe Islands (Yorifuji et al. 2011). However, it is possible that a beneficial effect of nutrients associated with high levels of maternal fish consumption could have confound the effects of low dose exposure to mercury and lead (Daniels et al., 2004; Oken et al., 2008). The lack of an interactive effect between Cd and Pb on neurodevelopmental outcomes, is consistent with two previous studies, one conducted in Korea (Kim et al. 2013) and the second based on data form the US National Health and Nutrition Examination Survey (Ciesielski et al. 2012). These findings support the need for research that examines the interactive effects of chemical element exposures on children's neurodevelopment and if these effects are influenced by maternal nutrient intake and status. Future research that applies recently developed statistical techniques such as Bayesian kernel machine regression and weighted quantile sum regression (Bobb et al., 2015; Valeri et al., 2017) could assist us in better

understanding the interactive effects of exposure to mixtures of chemical elements associated with ASGM activities on children's neurodevelopment.

Exposure to Pb, Hg, Cd and As has been associated with ASGM activities (Dooyema et al., 2012; Nyanza et al., 2014; Tirima et al., 2016) and one could expect that exposure levels would be correlated. However, the mode of environmental introduction and pathways of exposure differ for these chemical elements. For example, in Tanzania, As is a constituent of most of the gold ore (Nyanza et al. 2014) and is released into the environment during mechanical and chemical liberation of gold (Keshavarzi et al., 2012; Nyanza et al., 2014), whereas elemental Hg, which is used to extract gold from ore (UNEP 2013b), is released into the environment through the burning of Hg amalgam during the mining process (Nyanza et al. 2014).

Children living in ASGM areas have the potential to be continuously exposed to toxic chemical elements through breast milk, contaminated drinking water, air, and food sources (Bose-O'Reilly et al., 2010; Nyanza et al., 2014). These factors need to be considered in future studies as long-term exposure could have increasingly detrimental effects on neurodevelopment throughout childhood, adolescence and into adulthood (Grandjean et al., 2015; Jedrychowski et al., 2007; Oken et al., 2008)

In the present study, 31% of the infants who resided in ASGM areas were classified as displaying global neurodevelopmental impairment, which was associated with prenatal exposure to metallic or metalloid elements. This supports a need in ASGM communities for immediate action to reduce exposure to these elements among pregnant women and women of childbearing age. A first step could be the establishment of local environmental health and safety committees that could initiate and implement bylaws on safe mining tailing disposal and promote the adoption of cleaner mining technologies (i.e., reduction in the use of Hg in the mining process). In addition, environmental remediation around hot spots of metallic or metalloid elements has been shown to be successful in reducing exposure in other ASGM areas, such as in Zamfara state, Nigeria (Tirima et al. 2016).

The high rate of neurodevelopmental impairment among infants in ASGM communities also supports the need for early intervention to improve long term neurodevelopmental outcomes. Evidence from LMIC countries has shown that exposing infants to cognitively stimulating activities enriches their cognitive and social emotional competencies (Engle et al., 2007; Walker et al., 2007). One way of achieving this could be through the establishment of early childhood developmental spaces; dedicated spaces at a health facility or community playground where a trained community worker could work with caregivers on how they can best help their child grow and develop.

In Tanzania, the only ongoing health screening conducted for infants is weight checks, which are done on a monthly basis. No neurodevelopmental screening is conducted at health facilities and staff lack training in screening children for neurodevelopmental disabilities. Since the prevalence of neurodevelopmental impairment in areas with ASGM activities is significant, training staff at local health facilities to screen for significant impairments using commonly used and validated measures such as the Ten Question screen could be a first step in initiating and integrating neurodevelopmental screening services into local health facilities (Durkin et al. 1994).

This study has some limitations. First, all metallic and metalloid exposures were based on prenatal maternal concentrations. The actual amount of the chemical elements transferred to the fetus may vary from one woman to another depending on maternal health status including nutritional status. Second, we did not document early childhood illness. Some of the infants may have experienced a significant childhood illness prior to the neurodevelopmental assessment that could have moderated their respective performance. Further, frequent bouts of illness can reduce infants' opportunities to explore surrounding environments at critical periods for learning basic social and intellectual skills, which can be associated with lower scores on measures of neurodevelopment (UNICEF-WHO, 2012). It should be noted, however, that the infants who participated in this study were assessed when they were not ill or in need of any medical assistance. Individual children who were ill at the time of the scheduled assessment were sent for medical attention and rescheduled when they had completely recovered. Another limitation of this study is that we examined the total mercury exposure in blood spots without confirming the speciation. Blood mercury concentrations are generally accepted as valid biomarkers of methylmercury (Mergler et al. 2007), but without confirmation in this cohort we cannot be certain. Total mercury may include elemental mercury (Hg0) (also known as metallic mercury), inorganic mercury compounds (I-Hg) (primarily mercuric chloride), and organic mercury (primarily methylmercury (MeHg)). Different mercury species could have differing effects on children neurodevelopmental outcomes (Counter and Buchanan, 2004). Future research is needed in ASGM communities that examines mercury species in the surrounding environment, dietary items, and human blood and urine. Finally, we did not collect information on fish consumption among the pregnant women who participated in this study. However, previous research that has examined fish samples from the Rwamagasa area in Geita, Tanzania, has revealed Hg concentrations in fish tissue exceeding the WHO recommended limit for vulnerable groups (0.20 mg/kg) (Taylor et al. 2005). Therefore, the amount of fish that women consume could influence their level of exposure to Hg and their nutrient status, which in turn may interact to affect the neurodevelopmental outcomes of their infants. Future studies are needed in ASGM areas that untangle the associations among maternal prenatal fish consumption, Hg exposure and children's neurodevelopment.

To the best of our knowledge, this study is the first to examine prenatal exposure to neurotoxic metallic or metalloid elements and early neurodevelopmental outcomes in ASGM communities. Also, this is the first study to examine the effects of exposure to these elements and the interactions of these elements on neurodevelopmental outcomes in infants in areas with ASGM activities. The use of a validated tool developed to assess children in rural communities in Africa (i.e., the MDAT) provides robustness to the findings as the tasks and materials used were familiar to infants in our local setting. The internal consistency of the MDAT on all of the neurodevelopmental domains was also high. Further, the large sample size and direct measurement of prenatal concentrations of metallic and metalloid exposures using blood and urine provided added accuracy to the estimation of the prenatal levels of exposure as compared to indirect measures such as ecological assessment in drinking water.

In conclusion, infants born to women in areas with ASGM activities are at significant risk for neurodevelopmental impairment. Prenatal exposure to higher concentrations of Hg increased the risk of neurodevelopmental impairment among infants. Further, co-exposure to high concentrations of Hg and Pb, or Hg and As appeared to have negative potentiated effects on infants' neurodevelopment. Given the significant risk of neurodevelopmental impairment among infants living in ASGM areas of northern Tanzania, initiatives to reduce exposure to these elements and to improve early childhood development status are clearly warranted.

Funding

This study was supported by funding from the Canadian Institutes of Health Research through a Vanier Canada Graduate Scholarship to Elias C. Nyanza [grant number 244443], and a University of Calgary URGC Seed Grant (D Dewey, FP Bernier, University of Calgary, Calgary, Canada). Partial funding for the trace analysis of elements in a commercial laboratory was provided through an unrestricted General Research account (JW Martin, University of Alberta, Edmonton, Canada).

CRediT authorship contribution statement

Elias C. Nyanza: Conceptualization, Data curation, Formal analysis,

Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Writing - original draft, Writing - review & editing. **Francois P. Bernier:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing - review & editing. **Jonathan W. Martin:** Conceptualization, Formal analysis, Methodology, Supervision, Validation, Visualization, Writing - review & editing. **Mange Manyama:** Conceptualization, Formal analysis, Methodology, Supervision, Validation, Visualization, Writing - review & editing. **Jennifer Hatfield:** Conceptualization, Formal analysis, Methodology, Supervision, Validation, Visualization, Writing - review & editing. **Deborah Dewey:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors acknowledge the Department of Environmental and Occupation Health at the Catholic University of Health and Allied Sciences, Mwanza, Tanzania, the University of Calgary, Calgary, Canada, and the Regional Authorities in Mwanza and Geita, Tanzania for their collaboration and assistance with this study. The authors would like to thank all the women, infants and healthcare workers in Geita who participated in in this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.106104.

References

Barbone, F., Rosolen, V., Mariuz, M., Parpinel, M., Casetta, A., Sammartano, F., et al., 2019. Prenatal mercury exposure and child neurodevelopment outcomes at 18 months: results from the Mediterranean PHIME cohort. Int J Hyg Environ Health 222 (1), 9–21. https://doi.org/10.1016/j.ijheh.2018.07.011. PMID: 30057028.

Bobb, J.F., Valeri, L., Henn, B.C., Christiani, D.C., Wright, R.O., Mazumdar, M., et al., 2015. Bayesian kernel machine regression for estimating the health effects of multi-pollutant mixtures. Biostatistics 16 (3), 493–508. https://doi.org/10.1093/biostatistics/kxu058.

Bose-O'Reilly, S., McCarty, K.M., Steckling, N., Lettmeier, B., 2010. Mercury exposure and children's health. Curr Probl Pediatr Adolesc Health Care 40 (8), 186–215. https://doi.org/10.1016/j.cppeds.2010.07.002. PMID: 20816346.

Boucher, O., Burden, M.J., Muckle, G., Saint-Amour, D., Ayotte, P., Dewailly, É., et al., 2012. Response inhibition and error monitoring during a visual go/no-go task in Inuit children exposed to lead, polychlorinated biphenyls, and methylmercury. Environ Health Perspect 120 (4), 608–615. https://doi.org/10.1289/ehp.1103828. PMID: 22142904.

Chaudhuri, S.N., Butala, S.J., Ball, R.W., Braniff, C.T., 2009. Pilot study for utilization of dried blood spots for screening of lead, mercury, and cadmium in newborns. J Exposure Sci Environ Epidemiol 19, 298–316. https://doi.org/10.1038/jesee.2008.19.

Chen, W., Qian, L., Shi, J., Franklin, M., 2018. Comparing performance between log-binomial and robust Poisson regression models for estimating risk ratios under model misspecification. BMC Med Res Methodol 18 (1), 63. https://doi.org/10.1186/s12874018-0519-5. PMID: 29929477.

Ciesielski, T., Weuve, J., Bellinger, D.C., Schwartz, J., Lanphear, B., Wright, R.O., 2012. Cadmium exposure and neurodevelopmental outcomes in U.S. children. Environ Health Perspect 120 (5), 758–763. https://doi.org/10.1289/ehp.1104152. PMID: 22289429.

Counter, S.A., Buchanan, L.H., 2004. Mercury exposure in children: a review. Toxicol. Appl. Pharmacol 198 (2), 209–230. https://doi.org/10.1016/j.taap.2003.11.032.

Daniels, J.L., Longnecker, M.P., Rowland, A.S., Golding, J., The ALSPAC Study Team, 2004. Fish intake during pregnancy and early cognitive development of offspring. Epidemiology 15 (4), 394–402. https://doi.org/10.1097/01.ede.0000129514.46451.ce. PMID: 15232398.

Davidson, P.W., Cory-Slechta, D.A., Thurston, S.W., Huang, L., Shamlay, C.F., Gunzler, D., et al., 2011. Fish consumption and prenatal methylmercury exposure: cognitive and behavioral outcomes in the main cohort at 17 years from the Seychelles child development study. Neurotoxicology 32 (6), 711–717. https://doi.org/10.1016/j.neuro.2011.08.003. PMID: 21889535.

Davidson, P.W., Myers, G.J., Cox, C., Shamlaye, C.F., Marsh, D.O., Tanner, M.A., et al., 1995. Longitudinal neurodevelopmental study of Seychelles children following in utero exposure to methylmercury from maternal fish ingestion outcomes at 19 and 29 months. Neurotoxicology 16 (4), 677–688. PMID: 8714872. https://pubmed.ncbi.nlm.nih.gov/8714872/.

Debes, F., Weihe, P., Grandjean, P., 2016. Cognitive deficits at age 22 years associated with prenatal exposure to methylmercury. Cortex 74, 358–369. https://doi.org/10.1016/j.cortex.2015.05.017. PMID: 26109549.

Deroma, L., Parpinel, M., Togninc, V., Channoufi, L., Tratnik, J., Horvat, M., et al., 2013. Neuropsychological assessment at school-age and prenatal low-level exposure to mercury through fish consumption in an Italian birth cohort living near a contaminated site. Int J Hyg Environ Health 216 (4), 486–493. https://doi.org/10.1016/j.ijheh.2013.02.004. PMID: 23523155.

Dewey D, Konje E, Nyanza EC, Bernier FP, Manyama M. 2018. Chapter 17: Ethical Research in Global Child Health. Principles of Global Child Health: Education and Research. Laraque-Arena D, and Stanton BF (edt). America Academy of Pediatricts. ISBN (electronic) 978-1-61002-190-6. 299 – 320. Accessed on January 2020, Available at http://ebooks.aappublications.org/content/principles-of-global-child-health-education-and-research.

Dooyema, C.A., Neri, A., Lo, Y.C., Durant, J., Dargan, P.I., Swarthout, T., et al., 2012. Outbreak of fatal childhood lead poisoning related to artisanal gold mining in northwestern Nigeria, 2010. Environ Health Perspect. 120 (4), 601–607. https://doi.org/10.1289/ehp.1103965.

Durkin, M.S., Davidson, L.L., Desai, P., Hasan, Z.M., Khan, N., Thorburn, M.J., et al., 1994. Validity of the ten questions screen for childhood disability: results from population-based studies in Bangladesh. Jamaica and Pakistan. Epidemiology 5 (3), 283–289. https://doi.org/10.1097/00001648-199405000-00002. PMID: 7518697.

Engle, P.L., Black, M.M., Behrman, J.R., Mello, M.C., Gertler, P.J., Kapiriri, L., 2007. Strategies to avoid the loss of developmental potential in more than 200 million children in the developing world. Lancet 369 (9557), 229–242. https://doi.org/10.1016/S0140-6736(07)60112-3. PMID: 17240290.

Freire, C., Amaya, E., Gil, F., Fernadez, M., Murcia, M., Llops, S., et al., 2018. Prenatal co-exposure to neurotoxic metals and neurodevelopment in preschool children: the Environment and Childhood (INMA) Project. Sci Total Environ 621, 340–351. https://doi.org/10.1016/j.scitotenv.2017.11.273. PMID: 29190557.

Gladstone, M., Lancaster, G.A., Umar, E., Nirenda, M., Kayira, E., Van den Broek, N.R., et al., 2010. The Malawi Developmental Assessment Tool (MDAT): the creation, validation, and reliability of a tool to assess child development in rural African settings. PLoS. https://doi.org/10.1371/journal.pmed.1000273.

Golding, J., Gregory, S., Iles-Caven, Y., Hibbeln, J., Emond, A., Taylor, C.M., 2016. Associations between prenatal mercury exposure and early child development in the ALSPAC study. Neurotoxicology 53:215–222, PMID 26880023. https://doi.org/10.1016/j.neuro.2016.02.006.

Grandjean, P., Barouki, R., Bellinger, D.C., Casteleyn, L., Chadwick, L.H., Cordiers, S., et al., 2015. Life-long implications of developmental exposure to environmental stressors: new perspectives. Endocrinology 156 (10), 3408–3415. https://doi.org/10.1210/EN.2015-1350. PMID: 26241067.

Grandjean, P., Landrigan, P.J., 2014. Neurobehavioural effects of developmental toxicity. Lancet Neurol 13 (3), 330–338. https://doi.org/10.1016/S1474-4422(13)702. PMID: 24556010.

Gustin, K., Tofail, F., Vahter, M., Kippler, M., 2018. Cadmium exposure and cognitive abilities and behavior at 10 years of age: a prospective cohort study. Environ Int 113: 259–268, PMID 29459184. https://doi.org/10.1016/j.envint.2018.02.020.

Hamadani, J.D., Grantham-McGregor, S.M., Tofail, F., Nermell, B., Fangstrom, B., Huda, S.N., et al., 2010. Pre- and postnatal arsenic exposure and child development at 18 months of age: a cohort study in rural Bangladesh. Int J Epidemiol 39 (5), 1206–1216. https://doi.org/10.1093/ije/dyp369. PMID: 20085967.

Hamadani, J.D., Tofail, F., Nermell, B., Gardner, R., Shiraji, S., Bottai, M., et al., 2011. Critical windows of exposure for arsenic-associated impairment of cognitive function in pre-school girls and boys: a population-based cohort study. Int J Epidemiol 40 (6), 1593–1604. https://doi.org/10.1093/ije/dyr176. PMID: 22158669.

Harada, M., 1995. Minamata disease: methylmercury poisoning in Japan caused by environmental pollution. Crit Rev Toxicol 25 (1), 1–24. https://doi.org/10.3109/10408449509089885. PMID: 7734058.

Jedrychowski, W., Perera, F., Jankowski, J., Rauh, V., Flak, E., Caldwell, K.L., et al., 2007. Prenatal low-level lead exposure and developmental delay of infants at age 6 months (Krakow inner city study). Int J Hyg Environ Health 211 (3–4), 345–351. https://doi.org/10.1016/j.ijheh.2007.07.023. PMID: 17905657.

Jeong, K.S., Park, H., Ha, E., Hong, Y., Mi, Ha, Park, H., et al., 2015. Performance IQ in children is associated with blood cadmium concentration in early pregnancy. J Trace Elem Med Biol 30, 107–111. https://doi.org/10.1016/j.jtemb.2014.11.007. PMID: 25511909.

Keshavarzi, B., Moore, F., Rastmanesh, F., Kermani, M., 2012. Arsenic in the Muteh gold mining district, Isfahan. Iran. Environ Earth Sci 67, 959–970. https://doi.org/10.1007/s12665-012-1532-3.

Kim, Y., Ha, E.H., Park, H., Ha, M., Kim, Y., Hong, Y., et al., 2013. Prenatal lead and cadmium co-exposure and infant neurodevelopment at 6 months of age: the Mothers and Children's Environmental Health (MOCEH) study. Neurotoxicology 35, 15–22. https://doi.org/10.1016/j.neuro.2012.11.006. PMID: 23220728.

Kippler, M., Tofail, F., Hamadani, J.D., Gardner, R.M., Grantham-McGregor, S.M., Bottai, M., et al., 2012. Early-life cadmium exposure and child development in 5-

year-old girls and boys: A cohort study in rural Bangladesh. Environ Health Perspect 120 (10), 1462–1468. https://doi.org/10.1289/ehp.1104431. PMID: 22759600.
Landrigan, P.J., Goldman, L.R., 2011. Children's vulnerability to toxic chemicals: A challenge and opportunity to strengthen health and environmental policy. Health Affairs 20 (5), 842–850. https://doi.org/10.1377/hlthaff.2011.0151. PMID: 21543423.
Lanphear, B.P., Hornung, R., Khoury, J., Yolton, K., Baghurst, P., Bellinger, D.C., et al., 2005. Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. Environ Health Perspect. 113 (7), 894–899. https://doi.org/10.1289/ehp.7688.
Lauritzen, L., Brambilla, P., Mazzocchi, A., Harsløf, L.B., Ciappolino, V., Agostoni, C., 2016. DHA Effects in Brain Development and Function. Nutrients. 8 (1), 6. https://doi.org/10.3390/nu8010006.
D. Mergler H.A. Anderson L.H. Chan K.R. Mahaffey M. Murray M. Sakamoto et al. Methylmercury exposure and health effects in humans: a worldwide concern Ambio. 36 1 2007 3 11 10.1579/0044-7447(2007)36[3:meahei]2.0.co;2 PMID: 17408186.
Msoffe, C.U., Nyanza, E.C., Thomas, D.S.K., Jahnpour, O., Dewey, D., 2018. The sources and chemical content of edible soil sticks sold in markets in Tanzania: a cross-sectional analytical study. Environ Geochem Health 4 (2), 893–906. https://doi.org/10.1007/s10653-018-0185-x. PMID: 30203220.
Nyanza, E., Dewey, D., Bernier, F., Manyama, M., Hatfield, J., Martin, J., 2019b. Validation of dried blood spots for maternal biomonitoring of non-essential elements in an artisanal and small scale gold mining area of Tanzania. Env Tox Chem 38 (6), 1285–1293. https://doi.org/10.1002/etc.4420. PMID: 30900767.
Nyanza, E.C., Bernier, F.P., Manyama, M., Hatfield, J., Martin, J.W., Dewey, D., 2019. Maternal exposure to arsenic and mercury in small-scale gold mining areas of northern Tanzania. Env Res 173, 432–442. https://doi.org/10.1026/j.envres.2019.03.031. PMID: 30974369.
Nyanza, E.C., Dewey, D., Manyama, M., Martin, J.W., Hatfield, J., Bernier, F.P., 2020. Maternal exposure to arsenic and mercury and associated risk of adverse birth outcomes in small-scale gold mining communities in Northern Tanzania. Environ Int 137. https://doi.org/10.1016/j.envint.2019.105450.
Nyanza, E.C., Dewey, D., Thomas, D.S.K., Davey, M., Ngallaba, S.E., 2014. Spatial distribution of mercury and arsenic levels in water, soil and cassava plants in a community with long history of gold mining in Tanzania. Bull Environ Contam Toxicol 93 (6), 716–721. https://doi.org/10.1007/s00128-014-1315-5. PMID: 24923470.
Nyaradi, A., Li, J., Hickling, S., Foster, J., Oddy, W.H., 2013. The role of nutrition in children's neurocognitive development, from pregnancy through childhood. Front Hum Neurosci. 7, 97. https://doi.org/10.3389/fnhum.2013.00097. PMID: 23532379.
Oken, E., Radesky, J.S., Wright, R.O., Bellinger, D.C., Amarasiriwardena, C.J., Kleinman, K.P., et al., 2008. Maternal fish intake during pregnancy, blood mercury levels, and child cognition at age 3 years in a US cohort. Am J Epidemiol 167 (10), 1171–1181. https://doi.org/10.1093/aje/kwn034. PMID: 18353804.
Oken, E., Wright, R.O., Kleinman, K.P., Bellinger, D., Amarasiriwardena, C.J., Ha, Hu, et al., 2005. Maternal fish consumption, hair mercury, and infant cognition in a U.S. cohort. Environ Health Perspect 113 (10), 1376–1380. https://doi.org/10.1289/ehp.8041. PMID: 16203250.
Parajuli RP, Fujiwara T, Umezaki M, Watanabe C. 2013. Association of cord blood levels of lead, arsenic, and zinc with neurodevelopmental indicators in newborns: a birth cohort study in Chitwan Valley, Nepal. Environ Res 121, 45–51, PMID: 23164520, https://doi.org/10.1016/j.enres.2012.10.010.
Parvez F, Wasserman GA, Factor-Litvak P, Liu X, Slavkovich V, Siddique AB, et al. 2011. Arsenic exposure and motor function among children in Bangladesh. Environ Health Perspect 119(11):1665–1670, PMID: 21742576, https://doi.org/10.1289/eps1103548.
Rodriguez-Barranco M, Lacasana M, Aguilar-Garduno C, Alguacil J, Gil F, Gonzalez-Alzaga B, et al. 2013. Association of arsenic, cadmium and manganese exposure with neurodevelopment and behavioural disorders in children: a systematic review and meta-analysis. Sci Total Environ 03(47):454-455, PMID: 23570911, https://doi.org/10.1016/j.scitotenv.2013.03.047.
Roy A, Kordas K, Lopez P, Rosado JL, Cebrian ME, Vargas GG, et al. 2011. Association between arsenic exposure and behavior among first-graders from Torreon, Mexico. Environ Res 111(5):670–676, PMID: 21439564, https;//doi.org/10.1016/j.envres.2011.03.003.
Sanders AP, Claus Henn B, Wright RO. 2015. Perinatal and childhood exposure to cadmium, manganese, and metal mixtures and effects on cognition and behavior: a review of recent literature. Curr Environ Heal Rep 2(3):284-294, PMID: 26231505, https://doi.org/10.1007/s40572-015-0058-8.
Schnaas L, Rothenberg SJ, Flores MF, Martinez S, Hernandez C, Osorio E, et al. 2006. Reduced intellectual development in children with prenatal lead exposure. Environ Health Perspect 114(5):791-797; PMID: 16675439, https://doi.org/10.1289/ehp.8552.
Schulz C, Angerer J, Ewers U, Heudorf U, Wilhelm M. 2009. Revised and new reference values for environmental pollutants in urine or blood of children in Germany derived from the German Environmental Survey on Children 2003-2006 (GerES IV). Int J Hyg Environ Health 212(6): 637–647, PMID: 19589725, https://doi.org/10.1016/j.ijheh.2009.05.003.
Stata version 14.1Stata Corp LP®: College Station, TX.
Tamhane AR, Westfall AO, Burkholder GA, Cutter GR. 2016. Prevalence odds ratio versus prevalence ratio: choice comes with consequences. Stat Med 35(30):5730-5735, PMID: 27460748, https://doi.org/10.1002/sim.7059.
Taylor, H., Appleton, J.D., Lister, R., Smith, B., Chitamweba, D., Mkumbo, O., et al., 2005. Environmental assessment of mercury contamination from the Rwamagasa artisanal gold mining centre. Geita District Tanzania. Sci Total Environ 343 (1–3), 111–133. https://doi.org/10.1016/j.scitotenv.2004.09.042.
Tirima S, Bartrem C, von Lindern I, von Braun M, Lind D, Anka SM, et al. 2016. Environmental remediation to address childhood lead poisoning epidemic due to artisanal gold mining in Zamfara, Nigeria. Environ Health Perspect; 124: 1471–8, PMID: 26745642, https://doi.org/10.1289/ehp.1510145.
TNBS-MIS (Tanzania National Bureau of Statistics- Malaria Indicator Survey): 2017. Tanzania Demographic and Health Survey and Malaria Indicator Survey (TDHS-MIS) - Final Report 2015-16. Dar es Salaam, Tanzania. Accessed on October 2019, Available at https://dhsprogram.com/pubs/pdf/fr321/fr321.pdf.
Tofail, F., Vahter, M., Hamadani, J.D., Nermell, B., Huda, S.N., Yunus, M., et al., 2009. Effect of arsenic exposure during pregnancy on infant development at 7 months in rural Matlab. Bangladesh. Environ Health Perspect 117 (2), 288–293. https://doi.org/10.1289/ehp.11670.
Tolins M, Ruchirawat M, Landrigan P. 2014. The developmental neurotoxicity of arsenic: cognitive and behavioral consequences of early life exposure. Ann Glob Heal 80(4): 303-314, PMID: 25459332, https://doi.org/10.1016/j.aogh.2014.09.005.
Tyler CR, Allan AM. 2014. The effects of arsenic exposure on neurological and cognitive dysfunction in human and rodent studies: a review. Curr Environ Health Rep 1(2): 132-147, PMID: 24860722, https://doi.org/10.1007/s40572-014-0012-1.
UNEP (United Nations Environmental Programme). 2013. Global mercury assessment 2013: sources, emissions, releases, and environmental transport. UNEP. 42. Accessed on June 2019, Available at https://wedocs.unep.org/handle/20.500.11822/7984.
UNICEF-WHO (United Nations Children Fund and World Health Organization). 2012. Integrating early childhood development (ECD) activities into nutrition programmes in emergencies. Why, what and how. New York, NY: United Nations Children Fund. Accessed on January 2020 Available at http://www.who.int/mental_health/emergencies/ecd_note.pdf.
Valeri, L., Mazumdar, M.M., Bobb, J.F., Henn, B.C., Rodrigues, E., Sharif, O.I.A., et al., 2017. The joint effect of prenatal exposure to metal mixtures on neurodevelopmental outcomes at 20–40 months of age: evidence from rural Bangladesh. Environ Health Perspect 125(6):067015, PMID 28669934. https://doi.org/10.1289/EHP614.
Von Ehrenstein OS, Poddar S, Yuan Y, Mazumder DG, Eskenazi B, Basu A, et al. 2007. Children's intellectual function in relation to arsenic exposure. Epidemiology 18(1): 44–51, PMID: 17149142, https://doi.org/10/1097/01/ede/0000248900.65613/a9/.
Vyas, S., Kumaranayake, L., 2006. Constructing socio-economic status indices: how to use principal components analysis. Health Policy Plan 21 (6), 459–468. PMID: 17030551.
Walker, S.P., Wachs, T.D., Gardner, J.M., Lozoff, B., Wasserman, G.A., Pollitt, E., 2007. Child development: risk factors for adverse outcome in developing countries. Lancet 369 (9556), 145–157. https://doi.org/10.1016/S0140-6736(07)60076-2. PMID: 17223478.
Wasserman, G.A., Liu, X., Parvez, F., Ahsan, H., Factor-Litvak, P., Kline, J., et al., 2007. Water arsenic exposure and intellectual function in 6-year-old children in Araihazar. Bangladesh. Environ Health Perspect 115 (2), 285–299. https://doi.org/10.1289/ehp.9501. PMID: 17384779.
WHO (World Health Organization) 2010. Anthro for personal computers, version 3.2.2, 2011: Software for assessing growth and development of the world's children. Geneva: WHO. Available at, http://www.who.int/childgrowth/software/en. Accessed on, July 2018.
Wu, B.T., Dyer, R.A., King, D.J., Innis, S.M., 2013. Low fish intake is associated with low blood concentrations of vitamin D, choline and n-3 DHA in pregnant women. Br J Nutr. 109 (5), 936–943. https://doi.org/10.1017/S0007114512002103.
Xu, Y., Khoury, J.C., Sucharew, H., Dietrich, K., Yolton, K., 2016. Low-level gestational exposure to mercury and maternal fish consumption: associations with neurobehavior in early infancy. Neurotoxicol Teratol 54, 61–67. https://doi.org/10.1016/j.ntt.2016.02.002. PMID: 26876455.
Yorifuji, T., Debes, F., Weihe, P., Grandjean, P., 2011. Prenatal exposure to lead and cognitive deficit in 7- and 14-year-old children in the presence of concomitant exposure to similar molar concentration of methylmercury. Neurotoxicol Teratol 33 (2), 205–211. https://doi.org/10.1016/j.ntt.2010.09.00. PMID: 20868742.
Zeisel, S.H., 2006. Choline: critical role during fetal development and dietary requirements in adults. Annu Rev Nutr. 26, 229–250. https://doi.org/10.1146/annurev.nutr.26.061505.111156. PMID: 16848706.
Zou, G., 2004. A modified poisson regression approach to prospective studies with binary data. Am J Epidemiol 159 (7), 702–706. https://doi.org/10.1093/aje/kwh090. PMID: 15033648.